ABSTRACT OF THE DISCLOSURE

Quantitative and qualitative analysis of a nucleic acid analyte in a sample suspected to contain the nucleic acid analyte if achieved by first preparing a reaction mixture containing the sample and a known amount of an internal quantitation standard. At least a first aliquot of the reaction mixture is combined with a set of amplification reagents effective to amplify nucleic acids in the reaction mixture. The set of reagents includes at least one primer pair which is effective to amplify a first region of the nucleic acid analyte if present in the sample to produce a first amplified sample fragment and to amplify at least a portion of the internal quantitation standard to produce a control fragment. Amplification results in the formation of an amplification product mixture containing first amplified sample fragments and control fragments when the nucleic acid analyte is present in the sample, and only control fragments when the nucleic acid analyte is not present in the sample. The relative amounts of first amplified sample fragments and control fragments are analyzed to quantify the amount of nucleic acid analyte in the sample, and the sequence of the first amplified sample fragments is determined to assess the qualitative characteristics of any nucleic acid analyte. The internal quantification fragment is derived from the analyte nucleic acid by the incorporation of a plurality of sequence variations. These sequence variations include at least a first sequence variation effective to render the internal quantitation standard distinguishable from the first amplified sample fragment, and a second sequence variation effective to substantially eliminate the production of sequencing products from interaction of the internal quantitation standard and the first sequencing primer.